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Substrate and Irradiance Affect the Early Growth of the Endangered Tropical Tree Fern *Dicksonia sellowiana* Hook. (Dicksoniaceae)

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ABSTRACT.—*Dicksonia sellowiana* spores were cultivated in mineral solution. After 30 days, young gametophytes were transferred to different substrates: soil rich in organic matter; coxim: coconut fiber; sterilized red soil; sterilized red soil with the addition of organic compost, to determine the best substrate for gametophytes' and sporophytes' development. Red soil with the addition of compost was the best system for growth. When sporophytes were 1.5–2.0 cm in height, they were transferred to pots containing sterilized red soil with the addition of organic compost and kept in the field for 42 days, under 75, 50, 10 and 3% of irradiance. The longest frond height, frond quantity, fresh and dry mass, and RGR were observed in plants growing in 10% of irradiance. Plants kept under 100% irradiance died after 3 days, and under 50% and 75% irradiance they died gradually after 30 days. The fresh mass/dry mass ratio was higher at 3% and lower at 30% irradiance. The levels of chlorophyll a, chlorophyll b and total chlorophyll were higher in the plants grown at 3% irradiance. The levels of chlorophyll did not vary between 10 and 30% irradiance, with the exception of chlorophyll a, which was lower under 30% irradiance. The chlorophyll a/chlorophyll b ratio did not vary among treatments. This study provides information for the cultivation of *Dicksonia sellowiana* with special attention to conservation and sustainable management.

Dicksonia (Dicksoniaceae) is primarily a genus of tree ferns occurring in wet mountain forests, especially in the tropics (Tryon & Tryon, 1982). *Dicksonia sellowiana* Hook of Brazil is a terrestrial tree fern endangered due to the extensive harvesting in its habitat (IBAMA, 1997). The stem is usually massive and arborescent, about 10m tall or basally decumbent, bearing long, dense trichomes and many fibrous roots, which may sprout from the base or higher, almost to the apex. It occurs throughout Central America, from Venezuela to Colombia, and south to Bolivia, Paraguay, Uruguay and southeastern Brazil (Sehnem, 1978; Tryon, 1970, 1972; Tryon & Tryon, 1982). It grows at ca 1500–2500 m, sometimes up to 3500m, or especially in Brazil at lower elevations. In Brazil, it is known as "xaxim" or "xaxim bugio" and the trunks have been indiscriminately exploited through the commercialization of jars and substrate used in the production of ornamental plants, including fern cultivation (Sehnem, 1978). According to Santos (2002), in Paraná State, southern Brazil, about 1.1 million jars are produced monthly from approximately 140,000 plants of xaxim.

There is a lack of information on the biology of this species in the literature. A better understanding of the demography, ecology, physiology and life cycle could provide a basis for the development of a system of sustainable

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management that would contribute to the conservation of many endangered tree fern species (Bernabe et al., 1999). Spores of *D. sellowiana* need continuous white light and a temperature of 23 ± 2°C in order to achieve maximum germination (88%) seven days after sowing. The highest percentages of germination and the lower mean germination time for spores of *D. sellowiana* was observed for spores keep under 20 to 5% of light. The highest chlorophyll and soluble sugar contents were recorded in gametophytes cultivated for 49 days under 20 and 5% irradiance (Fillipini *et al.*, 1999; Renner and Randi, 2004). Spores of *D. sellowiana* remained viable after storage in liquid nitrogen (Rogge *et al.*, 2000). Borelli *et al.* (1990) cultivated *D. sellowiana* in the soil of "xaxim" trunks and observed sporophytes after six months' cultivation, but they did not mention the percentage of sporophyte emergence from gametophytes. They commented that fungal contamination was very high in all the treatments carried out.

Based on a few studies concerning *D. sellowiana* cultivation, the aim of the present study was to improve methods for its propagation from spores and to obtain some information about soil and light requirements for the early establishment of sporophytes in greenhouses or even in the field. Growth parameters analysed in this paper compare systems that might be convenient for the cultivation of *D. sellowiana*: numbers and lengths of fronds, levels of chlorophyll, fresh and dry mass, and relative growth rate. We expect that our results may provide a basis for the development of methods of propagation that will contribute to programs of management of this endangered species

Materials and Methods

Sporophylls of *D. sellowiana* were harvested in August 1999 in Urupema, in a fragment of the Atlantic Forest, situated between 27° 57′ 25″S and 49° 53′ 33″W in Santa Catarina state, Brazil. Sporophylls were air-dried in an oven at 30°C for three days on filter paper in order to induce dehiscence. The spores were removed and separated from debris by pressing the material through lens paper with a brush, and were then stored in glass jars under refrigeration at 7 ± 1°C.

Spores (960mg) were surface-sterilized using a 20% (v/v) solution of commercial bleach (2% of active chlorine) for a period of 30 min before filtering through sterile filter paper and washing several times with sterile distilled water. Spores were sown in 32 conical flasks containing 20 ml of Mohr's nutrient solution as presented by Dyer (1979) with the addition of 0.01% Benomyl. The flasks were plugged with two layers of autoclaved transparent commercial polypropylene film (7 × 7 cm) fixed with a rubber band. All procedures were carried out in a laminar hood. The spores were incubated under a 16-hour photoperiod (30 μmoles μmoles/sec/m^2) at 23 ± 2°C for 30 days, in January 2002. Subsequently, in February 2002, the young gametophytes were transferred to trays containing four types of substrates: substrate rich in organic matter used in gardens; coxim: substrate produced from the coconut fiber used as the substitute for the xaxim substrate; sterilized red soil; sterilized red soil with the addition of organic compost in the

TABLE 1. Analysis of substratum mineral composition (CIDASC-analysis number 07462/2003).

	Substrate			
	Garden soil	Coxim	Red soil	Red soil + compost
P	6.6	5.2	4.4	5.2
P (ppm)	+50	38.3	2.6	+50
K (ppm)	340	1204	95	450
Organic matter %	4.3	+10.0	0.8	0.9
Al (cmolc/l)	traces	0.3	2.2	traces
Ca (cmolc/l)	5.4	1.7	1.7	4.8
H^+, Al (cmolc/l)	2.48	1.89	8.79	3.90
N Total %	0.12	0.35	0.03	0.26
CEC (cmolc/l)	13.92	9.37	11.32	12.88

proportion of 3:1. The soil analysis was carried out in CIDASC (Companhia Integrada de Desenvolvimento Agrícola de Santa Catarina) and received the number 07462/2002 (Table 1).

The trays were covered with transparent film to avoid excessive water evaporation and plant dehydration. Substrate sterilization was carried out in a high power microwave oven for 10 minutes. The organic compost was produced from food waste at the University of Santa Catarina. The best substrate was the sterilized red soil with the addition of organic compost in the proportion of 3:1. When the first sporophytes were observed, 300 gametophytes were transferred to 6 trays (50 gametophytes in each tray) containing the same soil, in May 2002, with the objective of verifying sporophyte emergence curve and the percent sporophyte formation, and to obtain plants that were used later in growth analyses. Plants were kept in a growth room as described earlier until they were 1.5–2.0 cm in longest frond's length. After, the plants were transferred to small pots containing the same substrate and were kept in plastic trays covered with transparent film. They were then removed from the growth room and acclimated for 3 weeks. During acclimatization, the transparent film was removed from the trays, and gradually the pots were kept for 2 hours a day under canopy in field conditions.

Six trays containing 18 plants each were finally transferred to the field in September 2002 (Spring). Five of them were kept in 50 cm^3 boxes covered with black shade netting, which provided 3, 10, 50 and 75% total irradiance. The last was kept directly under the sun. The soil in the pots was kept hydrated throughout the test period to avoid the interference of water stress. Levels of irradiance inside the boxes were analyzed with a LICOR 250 quantameter, equipped with a PAR (photosynthetic active radiation) sensor (400 to 700 nm). On a typical March day, at midday, the photosynthetic photon flux density reaches 1400 μmoles/sec/m^2 in Florianópolis, SC, southern Brazil.

When the sporophytes were transferred to the boxes (Time 1) and after 42 days (Time 2), 3 blocks (with 3 plants) from each treatment were collected to measure the longest frond length and total frond fresh mass, dry mass, and

macroscopic leaf number. Chlorophyll contents were measured after 42 days utilizing nine plants from each light treatment and were quantified from absorbances at 645 and 633 nm according to Arnon (1949). Three 50 mg samples of fresh frond from each treatment were extracted in acetone and the absorbance was quantified with a GBC UV/VIS 916 spectrophotometer.

The RGR (Relative Growth Rate) was estimated as $(\text{Log } L_2 - \text{Log } L_1)/T_2 - T_1$ where Log is the natural logarithm, L2 is the frond length at Time 2 and L1 is the initial frond length when the sporophytes were transplanted to the boxes; T2 is Time 2 (42 days) and T_1 is the day of transplantation to the boxes (BERNABE *et al.* 2000). The RGR (Relative Growth Rate) was also estimated as $(\text{Logn } M_2 - \text{Logn } M_1)/(T_2 - T_1)$ where M is the dry mass and T is the time in days. Data were analyzed with Excel for Windows (Microsoft) and SAEG (1998) sofwares. The One way Anova, followed by the Multiple Range Test (Tukey $p < 0.05$) was used to compare data.

Results

Gametophytes were not able to develop in substrates rich in organic matter. In the coxim substrate, only filamentous gametophytes were observed after 245 days' culture. In the red soil substrate, the first sporophyte was observed after 180 days. On the other hand 30 days after transplantation into red soil substrate with the addition of organic compost, gametophytes were spathulate and the first sporophyte was observed 84 days after transplantation. After 245 days of cultivation, in the red soil substrate plus organic compost, 84.67% of gametophytes had produced sporophytes (Fig. 1).

The substrate rich in organic matter used in gardens had the highest pH and P and relatively high levels of K and Ca. The substrate coxim had low pH, a high level of P, the highest level of K and a low level of Ca. The red soil showed the lowest pH, a low level of P, a sufficient level of K, and a low Ca. Finally, the red soil with the addition of organic compost in the proportion of 3:1 had a low pH, higher level of P, and high levels of K and Ca. The percentage of N was highest in coxim substrate followed by red soil plus organic compost substrate. The cation exchange capacity was high in substrates rich in organic matter, followed by red soil and red soil plus organic compost. The levels of H and Al were highest in substrate red soil.

The highest values for frond length, number of fronds, fresh and dry mass (Fig. 2), RGR in dry mass, and RGR in height (Figs. 3a, 3b) were found at 10% irradiance. Plants kept at 100% irradiance died after 3 days, and ar 50% and 75% they died off gradually after 30 days. The fresh mass/dry mass ratio was highest at 3% and lowest at 30% irradiance (Fig. 3c). Total chlorophyll and chlorophyll a and b content (Figs. 3d, 4a, 4b) were highest in plants grown at 3% irradiance. Chlorophyll content was statistically similar at 10 and 30% irradiance, with the exception of chlorophyll a content, which was lowest at 30% irradiance. The chlorophyll a/ chlorophyll b ratio did not vary among treatments (Fig. 4c).

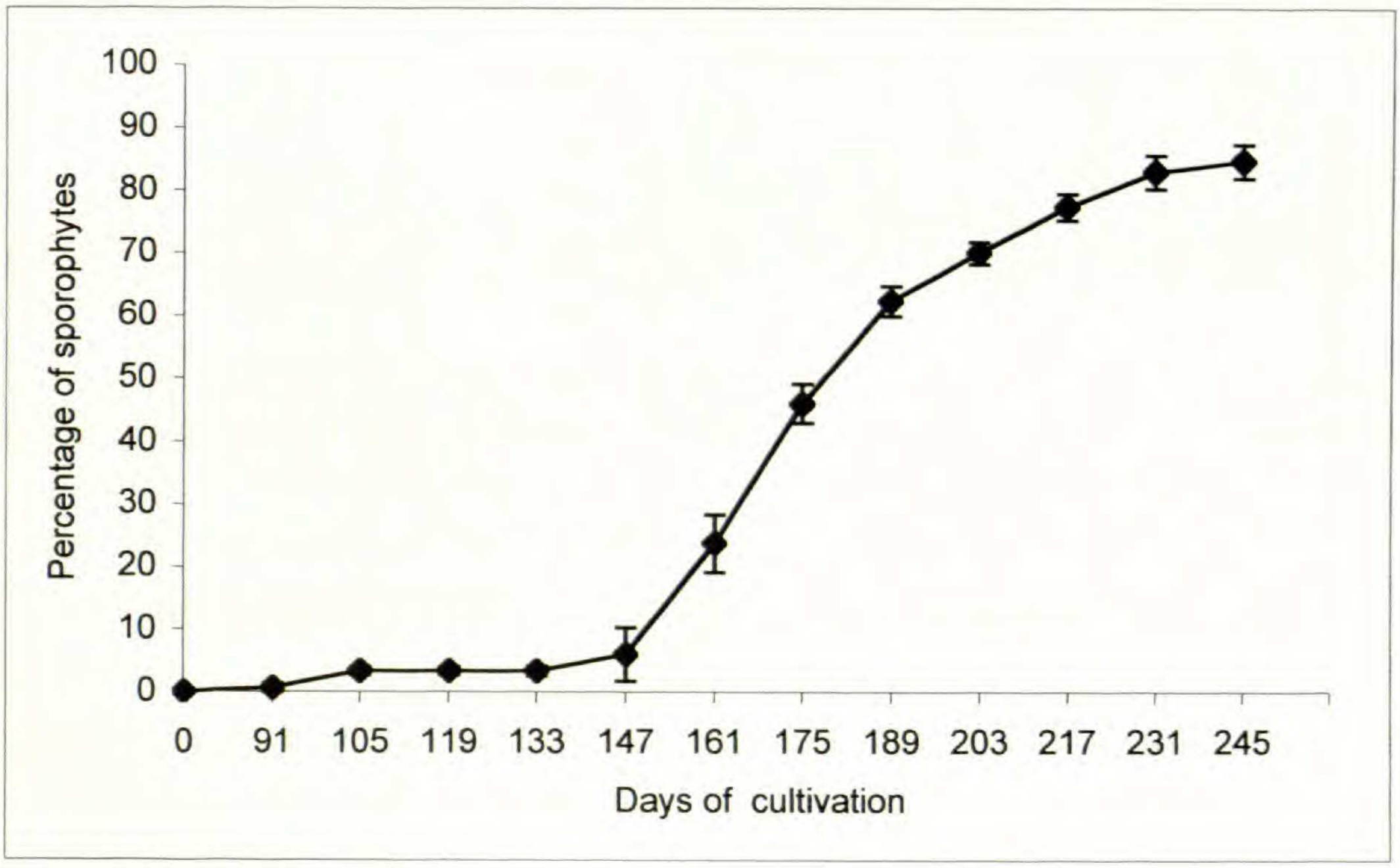

FIG. 1. Percentage of *Dicksonia sellowiana* sporophytes emerging from gametophytes cultivated in red soil with the addition of compost (3:1) in growth room at 25 ± °C and a 16-hour photoperiod. Bars are mean ± SD.

DISCUSSION

The red soil substrate with the addition of organic compost provided the best growth conditions for young plants of *D. sellowiana*. The first sporophytes were observed after 84 days of cultivation. On the other hand, Borelli *et al.* (1990) cultivated young plants of *D. sellowiana* in the soil of "xaxim" trunks and observed sporophytes only after six months' cultivation. They commented that the fungal contamination was very high in all the treatments carried out.

Dicksonia selowiana may prefer low pH and high levels of P, K, Ca and N as it develops only slowly in substrates rich in N and Al and poor in Ca, N and P (i.e. red soil alone). On the other hand, the plants did not develop at all on high pH media, as in the garden substrate. Although this species seems to prefer a reasonably high level of K, very high levels appear to be detrimental or even toxic. The coxim substrate has the highest level of K and was probably toxic to *D. sellowiana* development.

Edaphic parameters, including nutritional requirements, have been analyzed for some fern species to elucidate their habitats. Carlson (1979) compared the habitats of ten species of the *Dryopteris*; five species preferred acidic pH. Graves and Monk (1982) analyzed herbaceous fern composition together with several edaphic parameters in Georgia. Only *Polystichum acrostichoides* (Michx) Schott preferred acidic soil. *Athyrium pycnocarpon* (Spreng) Tidestrom grew in weakly acidic soil. *Athyrium thelypterioides* (Michx.) Desv. and

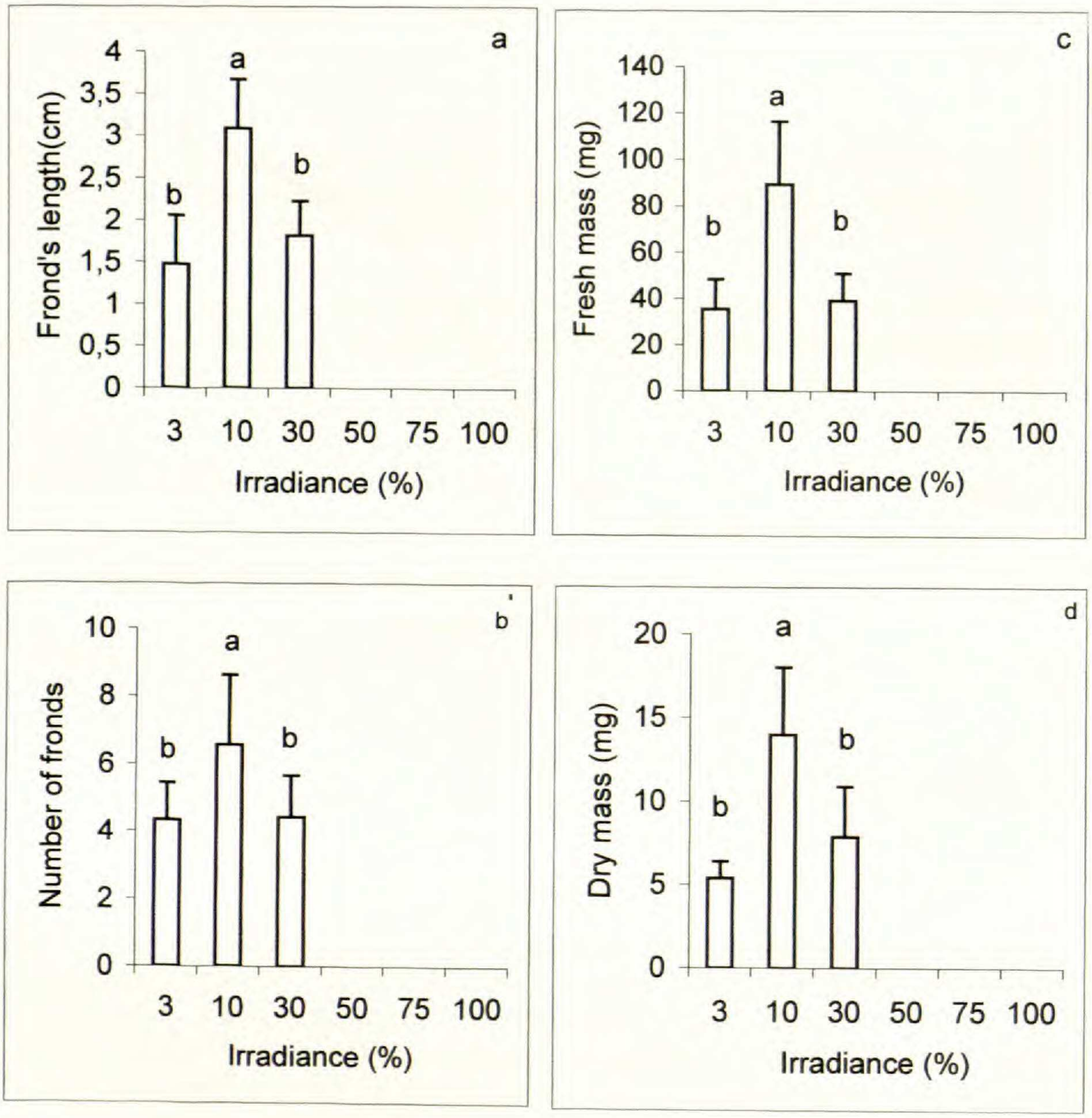

FIG. 2. Longest frond height (a), number of fronds (b), dry mass (c) and fresh mass (d) of sporophytes of *Dicksonia sellowiana* cultivated on red soil with the addition of compost, for 40 days at 3, 10, 30, 50, 75 and 100% irradiance (Florianópolis, Santa Catarina, Brazil). Letters denote statistical differences among treatments (Tukey , $p < 0.05$); bars with same letters are not different.

Cystopteris protusa (Weath.) Blasdell were considered generalists relative to pH requirements. Spores of *Ophioglossum palmatum* L. germinate in the dark and gametophytes seem to need a low pH for development (Whittier and Moyroud, 1993). Ranal (1995) suggests that fern distribution in São Paulo State, Brazil, is related to the level of mineral nutrition and soil pH. *Polypodium latipes* Langsd & Fisch is more abundant at low pH, high levels of aluminum, and lower levels of calcium. Others, such as *Microgramma squanulosa* (Kaulf.) Sota, are able to grow in a wide range of pH and are

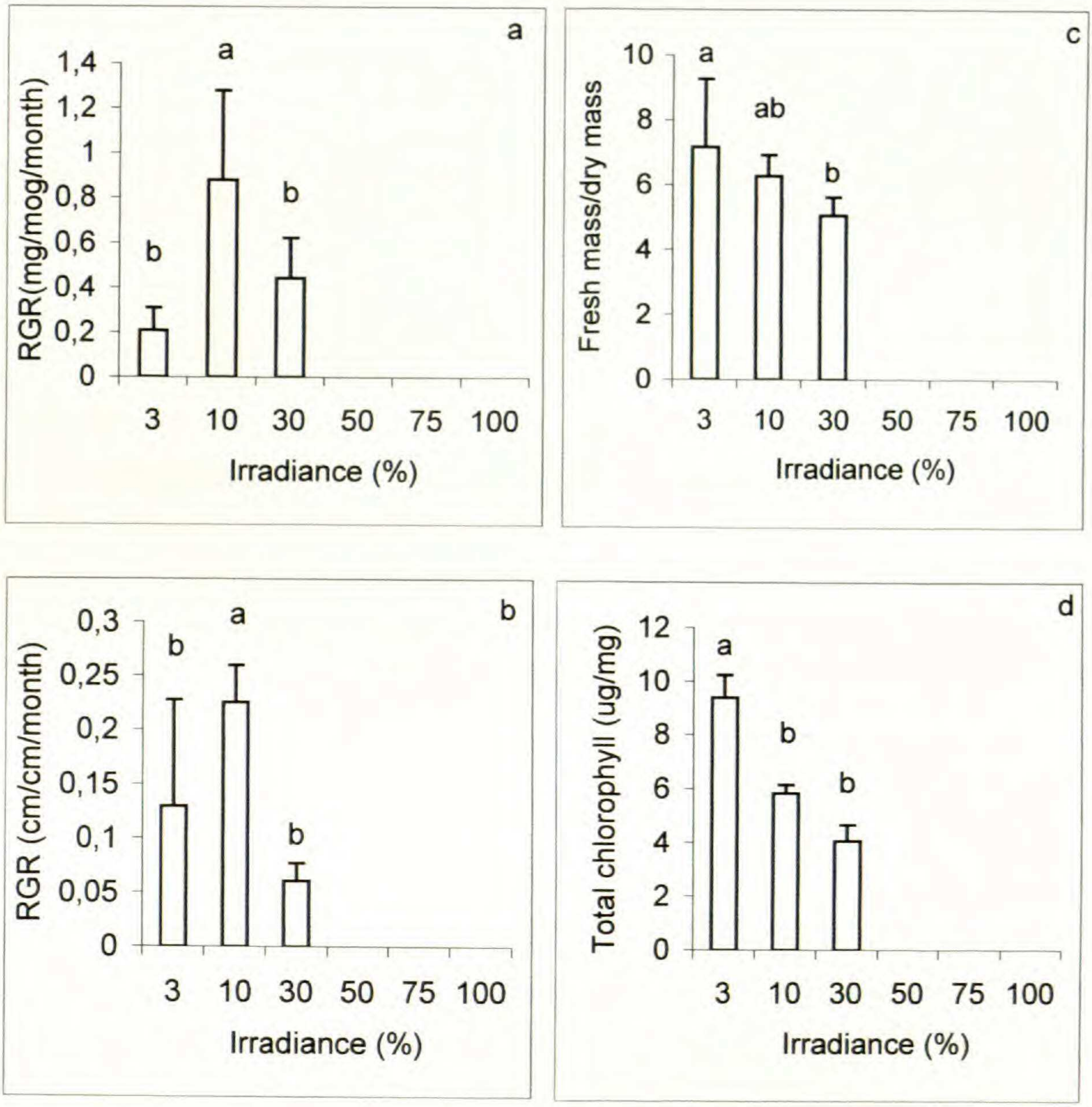

FIG. 3. Relative growth rate in dry mass (a), relative growth rate of the longest frond (b), fresh mass/dry mass ratio (c) and total chlorophyll (d) of sporophytes of *Dicksonia sellowiana* cultivated on red soil with the addition of compost for 40 days at 3, 10, 30, 50, 75 and 100% irradiance (Florianópolis, Santa Catarina, Brazil). Letters denote statistical differences among treatments (Tukey, $p < 0.05$); bars with same letters are not different.

considered generalists. Still others, such as *Pteris denticulata* Sw. and *Adiantopsis radiata* (L.) Fée, avoid high levels of calcium.

Young sporophytes of *D. sellowiana* did not survive at 50, 75 and 100% irradiance at sea level, in Florianópolis, SC, Brazil; they showed the greatest development when exposed to 10% irradiance. These data suggest that the light that reaches the ground of forests, 0.5 to 4% of sunlight (Chazdon & Fetcher, 1984), limits *D. sellowiana* development. The transient sun flecks across the canopy or the gaps could minimize the light scarcity on the level of

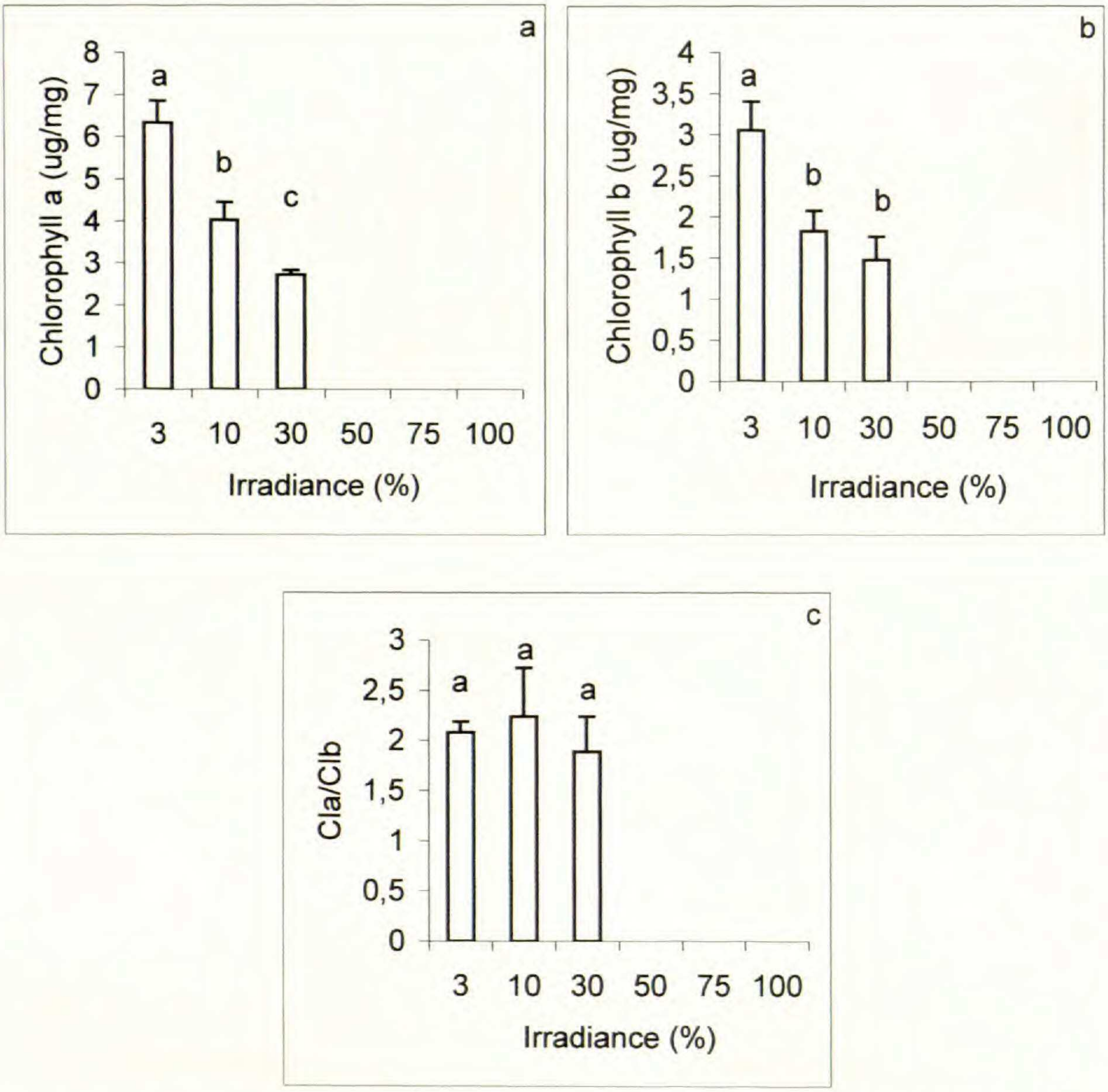

FIG. 4. Levels of chlorophyll a (a), chlorophyll b (b) and chlorophyll a/b ratio (c) of sporophytes of *Dicksonia sellowiana* cultivated on red soil with the addition of compost for 40 days at 3, 10, 30, 50, 75 and 100% light (Florianópolis, Santa Catarina, Brazil). Letters denote statistical differences among treatments (Tukey, $p < 0.05$); bars with same letters are not different.

the plants and provide a temporary enhancement of photosynthesis (Valladares *et al.*, 1997). On the other hand, high light intensities at sea level could induce photoinhibition of *D. sellowiana* which reduces photosynthetic efficiency, limiting plant growth and eventually causing the death of the plant (Demming-Adans and Adams, 1992; Sonoike, 1996; Kitao *et al.*, 2000). Bernabe *et al.* (1999) worked with three species of tree fern common to the Mexican montane cloud fragment, *Alsophila firma*, *Lophosoria quadripinnata* (Gmel.).C.Chr and *Sphaeropteris horrida* (Liebm.) Tryon and concluded that the forest edge was an appropriate habitat for the establishment of *Alsophila* and *Lophosoria* where PAR was nine times higher at the forest edge (160

μmoles/sec/m^2) than in the forest interior (18 μmoles/sec/m^2) on the date of observation. Tree fern species seem to vary in their tolerance to shade. *Cyathea pubescens* Mett.*ex* Kuhn growing in a Jamaican montane forest is considered a tolerant species because most individuals grow and produce spores in the forest shade. However, persistent sunflecks seem to be necessary for spore germination and probably, later, plants developing trunks will require higher irradiance for the establishment (Tanner, 1983). Arens and Baracaldo (1998), working in the Reserva Natural La Planada located between 1850 to 2300 m above sea level on the Pacific slope of the Andean Cordillera in Nariño, Colombia, observed that *Cyathea caracasana* (Kl.) Domin., *D. sellowiana*, and *L. quadripinnata* are an important part of the vegetation that colonizes open and abandoned pastureland areas in the Andes. In full sun, growth rates of *C. caracasana* are high (up to 2 cm/month) and individuals regularly produce spores. Plants are able to grow in the shade by the production of nearly vertical fronds with long stipes, apparently to place the photosynthetic surface into the canopy (Arens & Baracaldo, 2000). *Cyathea caracasana* performs best in full sun, but can persist under a closed canopy and was considered a habitat generalist (Arens, 2001).

The fresh mass/dry mass ratio, which reflects the level of the water, was highest in plants of *D. sellowiana* growing under lowest irradiance. Decreases in water content are common in high irradiance as a consequence of increases in the transpiration rates (Popma and Bonger, 1991; Niinemets and Kull, 1999; Dias-Filho, 1997). This could be another reason for the death of plants growing at 50, 75, and 100% irradiance. Contents of chlorophyll a, chlorophyll b and total chlorophyll were higher in plants of *D. sellowiana* grown at 3% irradiance than in plants grown at 10 and 30% irradiance. The increase in chlorophyll levels at lower irradiances is a characteristic pattern of light acclimatization of several species, and allows the leaves to absorb light even in the shade (Critchley, 1999). Sporophytes of *D. sellowiana* showed adjustment in the chlorophyll content under low irradiance, suggesting potentiality to increase light capture in such situations. Similar results were observed in the herbaceous fern *Adiantum raddianum* that showed an increase in chlorophyll when cultivated at low irradiances (Yeh & Wang, 2000). The chlorophyll a/chlorophyll b ratio did not differ among treatments. This ratio usually decreases in response to light reduction (Anderson et al., 1988; Tinoco-Ojangurem and Pearcy, 1995) because an increase in Photosystem II, richer in chlorophyll b than Photosystem I, is a common feature in plant acclimatization (Tinoco-Ojangurem and Pearcy, 1995). This plasticity was not observed in *D. sellowiana*. Data concerning light adjustments in the chlorophyll a/chlorophyll b ratio in ferns, were not found in the literature, but similar results were also observed for three angiosperms of the Atlantic Forest, *Cedrela fissilis* Vell., *Cecropia glazioui* Sneth and *Bathysa australis* (St Hil.) Hook. *ex*. Sch. (Duz, 2001). The RGR of *D. sellowiana* at 10% light was similar to the *Alsophila* RGR growing in the interior of forest of Mexico, which showed a RGR of 2.42 cm/cm/year (Bernabe et al., 1999), wheras in this work, *D. sellowiana* showed a RGR of 2.7 cm/cm/year or 0.225 cm/cm/month at 10% light.

Our study suggests that young plants of *D. sellowiana* prefer acidic pH and a substrate that is rich in mineral nutrition, but that growth was inhibited in coxim soil, which has the highest levels of K^+ and was delayed in the red soil that showed the lowest pH and a low level of P. Plants perform better under 10% irradiation. Probably, they do not develop well in the interior of the forest under very low irradiances nor at sea level under very high irradiance, in Santa Catarina State, south of Brazil. Sunflecks or gaps might provide relatively higher levels of light on the shaded floor of the forest, influencing the establishment of *D. sellowiana*. The information provided in this paper certainly will be useful for the development of programs for plant growth in greenhouses or even in its natural environment, as part of strategies to assist in its conservation.

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